Vol. 43 No. 8

BULLETIN

OF THE

TORREY BOTANICAL CLUB

AUGUST, 1916

The development of the embryo sac and embryo of Cooperia Drummondii*

MARGARET B. CHURCH

(WITH PLATES 22 AND 23)

The following study of the embryo sac and embryo of *Cooperia Drummondii* was undertaken with the idea that a further knowledge of the embryo sac of the Amaryllidaceae would doubtless be of value and interest, since little or no work of this kind has been attempted recently in connection with this family.

The geographical range of *Cooperia Drummondii* extends from the prairies of southern Kansas southwest into northern Mexico and as far west as New Mexico. The leaves of a mature plant are glabrous, the very narrow grass-like blades arising from coated, subglobose bulbs which are six to eight inches below the surface of the ground. The roots are coarse and unbranched except when injured at the tip. The flower scape varies from pale green at the base to yellow at the perianth. Its veins are a distinct rose color. The flower at the time of opening is creamy white with the tips of the lobes of the salverform perianth sometimes rose-colored. It becomes pure white when in full bloom and on withering gradually changes to deep dull rose. No difficulty was encountered in obtaining seed in the greenhouse from self-pollinated flowers.

Cooperia Drummondii is popularly known as "Rain Lily," because the flower scapes shoot up and bear blooms very rapidly

[The BULLETIN for July (43: 343–396. *pl. 21*) was issued August 12, 1916.]

* Contribution from the Botanical Laboratory of Brown University.

after a heavy shower of rain. In contrast it is worth noting that *Habranthus* [*Zephyranthes*] *gracilifolius* β *Boothianus* breaks the sheath three days before the flower opens (Lindley, 7). The flowering period of *Cooperia Drummondii* in the vicinity of Austin, Texas, usually includes the last week in April and the first week in May.

The material for the study of the development of the embryo sac was furnished to the writer by Professor H. H. York, who collected it on the campus of the University of Texas. For the study of the development of the embryo, bulbs were secured from the above mentioned place, and were grown in the greenhouse at Brown University. Flemming's weaker and stronger solutions, chromo-acetic acid, alcoholic and aqueous picro-acetic acid were used in general for killing and fixation. Aqueous picro-acetic gave the best results in the case of the embryo sac and the mature embryo. The following additional solutions were tried in an attempt to prevent the shrinking of the immature embryo during the process of killing and fixation: (1) 2 c.c. of a one per cent. aqueous solution of acetic acid, 11 c.c. of a one per cent. aqueous solution of osmic acid and 11 c.c. of water for fifteen minutes; (2) a one per cent. aqueous solution of osmic acid for fifteen minutes; (3) a one per cent. aqueous solution of osmic acid for three minutes, followed by twenty-four hours in Schaffner's chromo-acetic; (4) a solution of osmic acid and acetic acid in the proportions mentioned above for three minutes, followed by Schaffner's chromo-acetic for twenty-four hours; and (5) a two per cent. aqueous solution of acetic acid for twenty-four hours. The two per cent. aqueous solution of acetic acid proved to be a perfect fixing agent for the immature embryos, especially if the individual ovules were dissected out from the ovary immediately before fixing. Dehydration and imbedding were carried on in the usual way, except in the case of embryos large enough to be dissected out of the ovule. Here the laying of the material in a certain position in order that sectioning might be in the right plane was facilitated by staining the embryos *in toto* by means of a dip into erythrosin before dehydration was completed, and the subsequent use of the binocular microscope and substage illumination as will be described. The binocular microscope was unscrewed

from its stand and placed upon a ground glass plate below which in a convenient compartment was an electric light bulb. A glass plate, bearing the imbedding ells, was placed on the arms of the stage. The light from the electric bulb readily made visible the gross structure of the embryo, so that the orientation of the object could be positively determined. Care was taken not to overheat the lenses of the binocular by leaving the electric bulb lighted for too long a period. The imbedding was done therefore quite rapidly. Serial sections 10–12 μ in thickness were cut. Delafield's hematoxylin; Haidenhain's iron-alum haematoxylin with fuchsin, erythrosin or Orange G; thionin with erythrosin; and Flemming's triple stain were used. The last gave the best results.

Very early in the development of the ovule, the primitive archesporial cell, which is hypodermal in origin, may be distinguished from the surrounding somatic tissue by its size, its granular content and its prominent nucleus (FIG. 1). Two hypodermal cells in one ovule were noted in five examples out of one hundred and forty-six ovules examined (FIG. 2). It was not possible to follow the subsequent development. Guignard (4) reported this condition in *Ornithogalum pyrenaicum*, and similar instances have been found in an increasing number of the Liliaceae. See Coulter and Chamberlain (1), Lechmere (6), and McAllister (8, 9). At this time the integuments of the ovule are not visible. The archesporial cell develops at the expense of the surrounding nucellus into a large oblong cell, which stands with its longer axis parallel to that of the ovule (FIG. 3). No tapetal cells were observed. The absence of a parietal tissue occurs among some of the close relatives of the Amaryllidaceae, as in *Allium*, *Hemerocallis*, *Lilium*, and *Erythronium* of the Liliaceae; and in *Sisyrinchium iridifolium* and *Iris stylosa* of the Iridaceae (Coulter and Chamberlain, 1).

Megaspore formation takes place in the way usual for the Liliaceae. The archesporial cell becomes directly the one-celled stage of the embryo sac (FIG. 3). It was not possible to follow out the behavior of the chromosomes in any of the divisions of the embryo sac nuclei. Since the mode of development of the embryo sac of *Cooperia Drummondii* is similar to that of *Ery-*

thronium (Schaffner, 11), *Lilium* and *Tulipa Gesneriana* (Treub and Mellink, 12) and other Liliaceae, we may for the present assume that the reduction division occurs with the division of the one-celled stage and is completed when the four-nucleate embryo sac is formed (FIGS. 4, 5). By a division of the four nuclei the eight-nucleate sac results (FIG. 6).

At the time of fertilization the two polar nuclei are in the chalazal half of the sac, the antipodals near the chalaza, and the egg cell and the synergids opposite the micropyle (FIG. 6). The polar nuclei have a very thin protoplasmic content, although their nucleoli stain heavily. The three antipodals are at first arranged in a pyramid whose apex points toward the micropyle. Just before fertilization they become rearranged in a row, parallel with the longitudinal axis of the chalazal end of the embryo sac; while after fertilization they generally move again, this time to form a pyramid with its apex directed toward the chalaza. The antipodals stained very deeply in all of the combinations of stains that were used. This may be indicative of disintegration. The synergids have dense but vacuolated protoplasm. The egg cell is large and pear-shaped with a large round nucleus. It is directly in front of the micropyle. The dense protoplasm of the egg is less vacuolated than that of the synergids.

The nucellus at the time of fertilization has been largely absorbed or digested except for a thin layer of cells about the micropylar portion of the sac and a small mass of cells located at the chalaza and almost wholly deprived of protoplasm. The antipodals show a marked tendency to enlarge, and in numerous instances increase so as to almost equal the egg cell in size. This tendency toward enlargement persists even after fertilization, when the partial development of the embryo has taken place. Ordinarily, however, the antipodals have almost disintegrated when the embryo has become two-celled. The two polar nuclei, which are in close contact at maturation, fuse at the time of fertilization. A few examples of triple fusion were observed (FIG. 8). Ernst (2) has observed this condition in *Paris quadrifolia*. Further, it has been observed in other Liliaceae: as in *Trillium grandifolium;* in species of *Lilium*, of *Fritillaria*, and of *Tulipa;* and in *Endymion nutans* (Coulter and Chamberlain, 1). The synergids disappear at fertilization.

Following fertilization the endosperm nucleus divides, and by subsequent mitotic divisions a number of free nuclei, apparently without nuclear walls, are formed (FIG. 17), which are imbedded in the cytoplasm lining the wall of the sac and surrounding the embryo. Free nuclei are found in *Alisma Plantago* (Schaffner, 10), *Leucojum vernum* (Hofmeister, 5), and *Xyris indica* (Weinzieher, 13). Eventually these nuclei become separated by cell walls, which are at first laid down so as to include several nuclei in each lumen (FIG. 18). Later these multinucleate cells divide in such a manner that a single nucleus becomes enclosed within a lumen. The cells divide by amitosis after the walls are laid down. Several cases have been noted where endosperm formation was lacking. In connection with endosperm development in *Cooperia Drummondii* the description of endosperm formation in *Leucojum vernum* as given by Hofmeister (5) is of interest: ". . . the kernels in the inner fluid of the embryo sac increase in size and number; most of them lie now against the wall of the embryo sac; . . . they unite with the cells which fill up the space of the embryo sac. The lower part of the nucellus is pressed continually outward by the growing endosperm as the seed ripens."

At first it was impossible to kill and fix the early stages of the embryo. This difficulty was due to the fact that a suitable killing and fixing agent which would not cause great shrinkage of the protoplasm in these particular stages was not known. Therefore free hand sections or sections cut on a freezing microtome were used. The working out of the anatomical details of the developing embryo becomes possible with the use of a two per cent. acetic acid in water solution as a killing and fixing agent.

After fertilization the oöspore continues to occupy the same position as the oösphere. It enlarges (FIG. 7) and soon divides by a transverse wall (FIG. 9). Soon after the first division of the oöspore, the two-celled proembryo is in contact with the nucellus at the micropylar end of the embryo sac, and a little later is firmly attached at the same point. The basal cell of the proembryo develops into a suspensor, while the free terminal cell gives rise to the embryo proper (FIG. 10). The second cell division of the proembryo occurs in the basal cell, resulting in the

formation of a two-celled suspensor. Further cell divisions in the suspensor have not been observed. A number of examples were found where the suspensor consisted of but a single cell. Following the division of the basal cell, the free terminal cell divides first in a plane parallel with the longitudinal axis of the proembryo, forming the two-celled embryo (FIG. 11). The four-celled stage results from radial walls perpendicular to the first (FIG. 12).

From the four-celled stage by repeated division arises a spherical mass of cells (FIGS. 13, 14), on one side of which develops a niche or indentation (FIG. 15). This niche is the point where the shoot primordium will arise, while the cotyledon differentiates off from the rounded top, and the hypocotyl from the cells just above the suspensor of this immature embryo. In one instance this niche in an early stage was covered with a thin cell-like transparent membrane (FIG. 16). Later stages are shown in FIGS. 19 and 20.

The structure of the mature embryo may be seen in FIGS. 22 and 23. In relief a scarcely protruding pocket protects the leaf primordia by surrounding their outer surface as they lie against the base of the cotyledon. This is the "cotylar" sheath, corresponding to the sheath of a monocotyledonous leaf. This sheath has no vascular system and is very simple in structure. The long spindle-shaped cotyledon is succulent and projects into the mass of the endosperm (FIG. 21), where like a haustorium it absorbs food for the development of the embryo by means of a thin-walled row of parenchymatous cells running parallel with the longitudinal axis of the embryo. This strong development of the cotyledon is assumed to be in itself indicative of richness of endosperm development, and is, according to Goebel (3), characteristic of plants such as *Leucojum* and *Narcissus*.

The suspensor, varying in the number and arrangement of its cells, still holds the embryo in position at maturity.

The number of leaf primordia developed at the time the seed is ripe depends on the individual embryo, influenced as it is by various conditions of internal and external factors. No cases have been noted where the first leaf is undeveloped at the end of the same period, but the development and size attained by such leaf primordia in a mature embryo is not at all constant. The

leaf base of *Cooperia Drummondii*, like that of *Leucojum* and *Narcissus* (Goebel, 3), is closed. The primary root arises from the base of the hypocotyl and developing downward is protected by a well-developed root cap even as early as at the maturity of the seed.

In conclusion it may be of interest to contrast Hofmeister's description (5) of embryo sac and embryo development in *Amaryllis longiflora* with that of *Cooperia Drummondii*. They are almost entirely dissimilar. Hofmeister states that *A. longiflora* (*Hippeastrum solandriflorum*) is the only member of the Amaryllidaceae having irregular megasporangium formation. Just what he had in mind the writer does not dare to say, as no figures are given and his interpretation of the embryo sac was not that of present-day botanists. In regard to *Cooperia Drummondii* the statement may again be made that the embryo-sac development is without doubt regular, as compared with its near relatives.

In *Amaryllis longiflora* endosperm formation is sparing. The endosperm cells are displaced by the embryo before they become united into a compact tissue; while *Cooperia Drummondii*, as has been shown, has a firm, abundant and well-developed endosperm tissue. The embryo of *Amaryllis longiflora* is spindle-shaped in the region of the root and plumule but the cotyledon is bent and consequently knee-shaped, where that of *Cooperia Drummondii* grows straight upward forming a regular, spindle-shaped body except in individual instances where too much externalpres sure is exerted by earlier developed or more rapidly growing ovules.

This paper represents in part work undertaken by the author during the years 1912–1914 for the degree of Master of Arts at Brown University. The writer begs to acknowledge the assistance given by Professor Harlan H. York, of the Botanical Deaprtment of Brown University, and the courtesies of all others who may have made any helpful suggestions whatsoever.

LITERATURE CITED

1. **Coulter & Chamberlain.** Morphology of the Angiosperms. New York. 1903.
2. **Ernst, A.** Chromosomenreduction, Entwickelung des Embryosackes und Befruchtung bei *Paris quadrifolia* L. and *Trillium grandiflorum* Salisb. Flora **91**: 1–46. *pl. 1–6.* 1902.

3. **Goebel, K.** Organography of plants. English Translation. Oxford. 1900–05.
4. **Guignard, L.** Recherches sur le sac embryonaire des Phanérogames Angiospermes. Ann. Sci. Nat. Bot. VI. **13**: 136–199. *pl. 3–7.* 1882.
5. **Hofmeister, W.** Neuere Beobachtungen über Embryobildung der Phanerogamen. Jahrb. Wiss. Bot. **1**: 82–188. *pl. 7–10.* 1858.
6. **Lechmere, A.** Two embryo-sac mother-cells in the ovule of *Fritillaria*. New Phytol. **9**: 257–259. *f. 19.* 1910.
7. **Lindley, J.** [*Habranthus gracilifolius β Boothianus.*] Bot. Reg. *pl. 1967.* 1837.
8. **McAllister, F.** The development of the embryo sac of *Smilacina stellata*. Bot. Gaz. **48**: 200–215. *pl. 15.* O 1909.
9. —— On the cytology and embryology of *Smilacina racemosa*. Trans. Wisconsin Acad. **17**: 599–660. *pl. 56–58.* 1913.
10. **Schaffner, J. H.** The embryo-sac of *Alisma Plantago*. Bot. Gaz. **21**: 123–132. *pl. 9, 10.* Mr 1896.
11. —— A contribution to the life history and cytology of *Erythronium*. Bot. Gaz. **31**: 369–387. *pl. 4–9.* Je 1901.
12. **Treub, M. & Mellink, J. F. A.** Notice sur le développement du sac embryonaire dans quelques angiospermes. Archiv. Néerlandaises Sci. **15**: 452–457. *pl. 9, 10.* 1880.
13. **Weinzieher, S.** Beiträge zur Entwicklungsgeschichte von *Xyris indica* L. Flora **106**: 393–432. *pl. 6–7 + f. 1–10.* 1914.

Explanation of plates 22 and 23

All figures except where noted are Zeiss camera lucida drawings from microtome sections. A Leitz compound microscope was used for all drawings. In some cases the scale of magnification is designated; in other cases the ocular and objective combinations are given.

FIG. 1. Archesporial cell, × 310.

FIG. 2. Ovule with two archesporial cells, × 300.

FIG. 3. Uninucleate embryo sac, × 310.

FIG. 4. Two-nucleate embryo sac, × 200.

FIG. 5. Four-nucleate embryo sac, × 110.

FIG. 6. Eight-nucleate embryo sac, × 150.

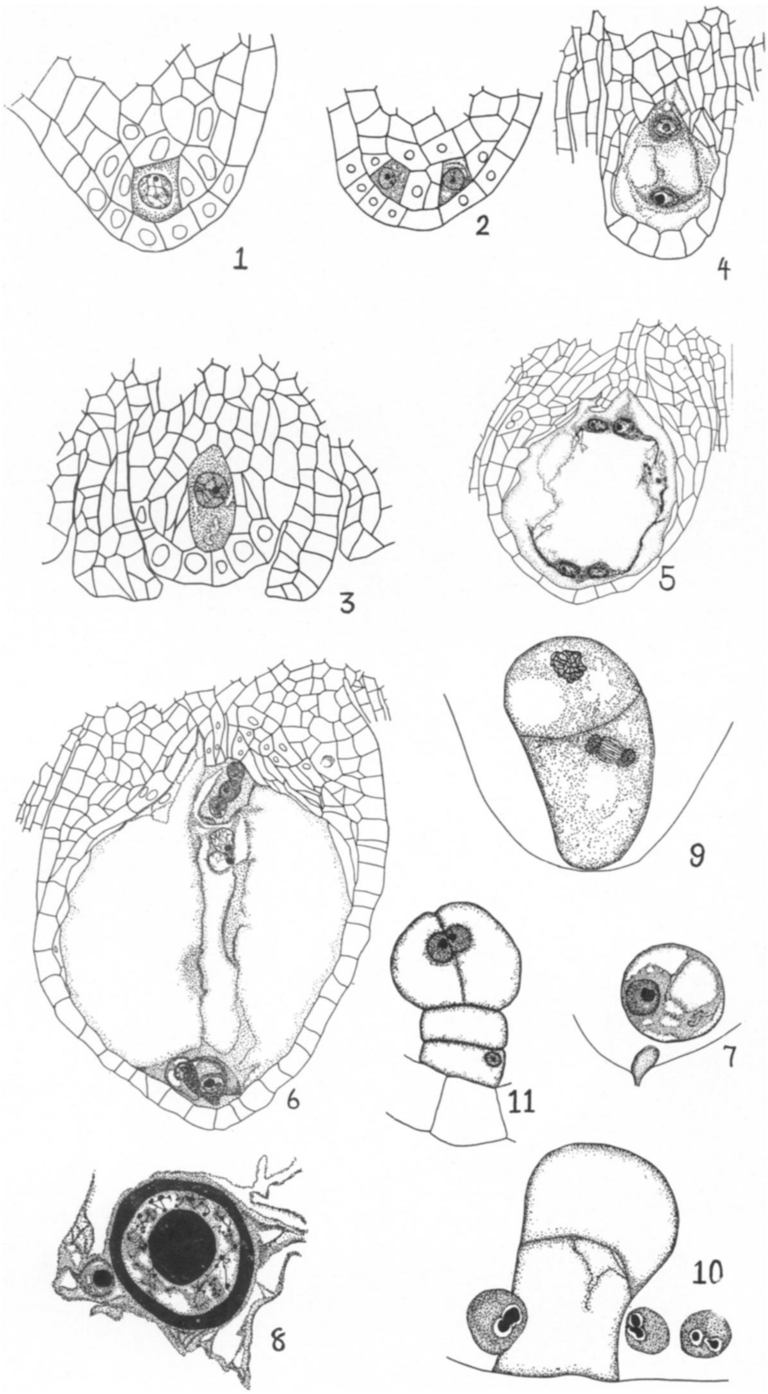
FIG. 7. Fertilized egg and pollen tube nucleus from fresh material; 1 ocular, 7 objective.

FIG. 8. Fused polar nuclei and pollen tube nucleus, × 600.

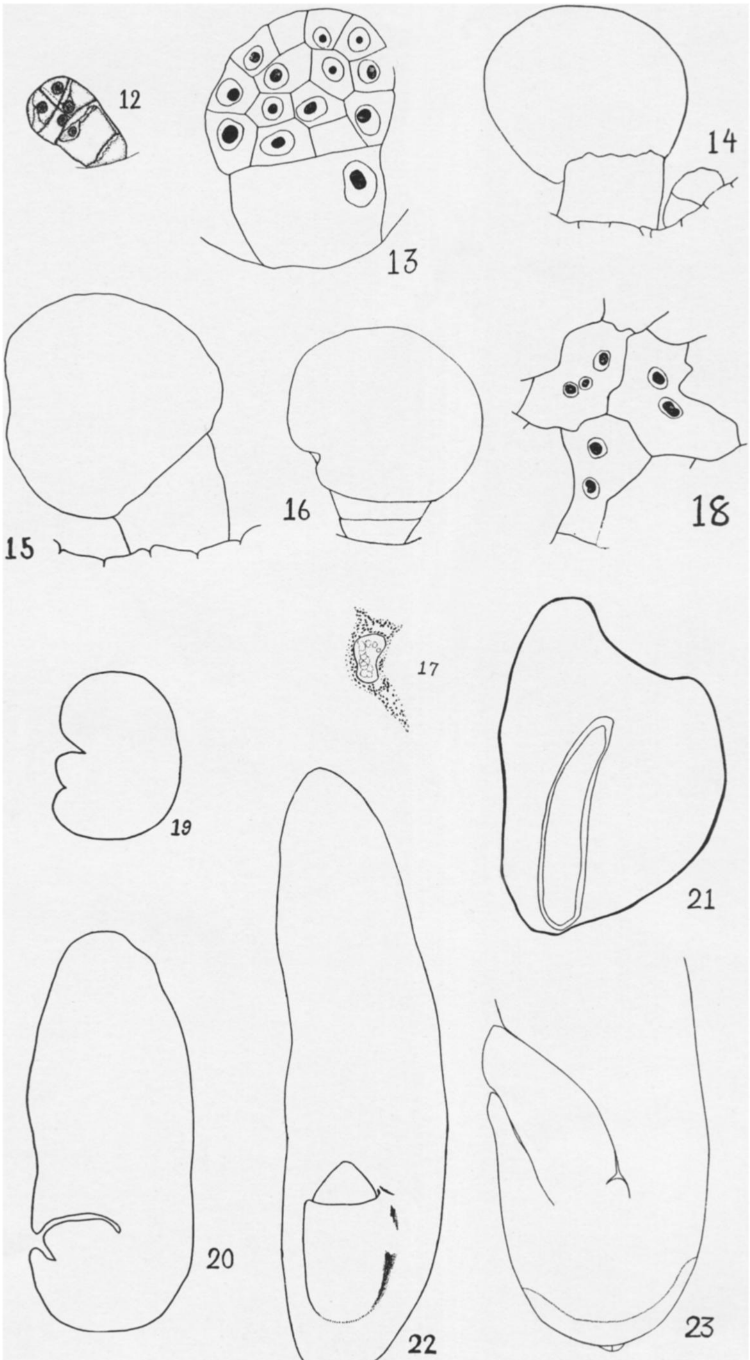
FIG. 9. Proembryo, two-celled and as yet unattached; 4 ocular, 7 objective

FIG. 10. One-celled embryo with suspensor and endosperm, from free-hand section of fresh material; 4 ocular, 7 objective.

FIG. 11. Two-celled embryo, from fresh material cut on freezing microtome, × 310.



CHURCH: COOPERIA DRUMMONDII



CHURCH: COOPERIA DRUMMONDII

FIG. 12. Four-celled embryo, from fresh material cut on freezing microtome, $\times$ 130.

FIG. 13. Longitudinal section through young embryo, from fresh material cut on freezing microtome, $\times$ 150.

FIG. 14. Outline of young embryo and suspensor with endosperm at one side, from free-hand section of fresh material; 4 ocular, 7 objective.

FIG. 15. Outline of embryo with suspensor, showing niche where growing point is developing, from free-hand section of fresh material; 4 ocular, 7 objective.

FIG. 16. Outline of embryo with suspensor, showing further differentiation of a growing point over which is a transparent cell-like membrane; 4 ocular, 7 objective.

FIG. 17. Free nucleus from endosperm; cytoplasm in which nucleus is imbedded not shown in drawing. Oil immersion.

FIG. 18. Cell walls being laid down between free nuclei of endosperm; walls warped by plasmolysis; diagrammatic, $\times$ 60.

FIG. 19. Outline of embryo, showing differentiation of root, sheath, plumule, and cotyledon regions, $\times$ 70.

FIG. 20. Outline of immature embryo, $\times$ 60.

FIG. 21. Diagram of mature seed, showing seed coats, endosperm, and embryo fitting loosely in the endosperm.

FIG. 22. Mature embryo, dissected out of ovule; 2 ocular, 1 inch Beck objective.

FIG. 23. Longitudinal section through mature embryo, $\times$ 150.